NOVEL COATING RESIN PLATE

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Abstract of **JP2002060671**

PROBLEM TO BE SOLVED: To provide a resin plate which efficiently and stably immobilize nucleic acid, peptide, protein, antibody, etc., and is suitable for processing, such as chipping. SOLUTION: This resin plate, used for immobilizing a biological material, is a resin substrate coated with a coating resin having at least one selected from among functional groups having positive charge, functional groups having tertiary amino groups, functional groups capable of forming covalent bonds with amino groups in a biological material, hydroxyl group, sulfo group, and phosphoric acid group or is a cured article of the coating resin.

FULL CONTENTS

[Claim(s)]

[Claim 1] Are the resin board used for fixation of living thing material, and [this resin board] The functional group which has positive charge, the functional group which has the third class amino group, the amino group in living thing material and the functional group which forms a covalent bond, The resin board for living thing material fixation characterized by being what a resin base material is coated with the coating resin which has at least one or more functional groups chosen from the group which consists of a hydroxyl group, a sulfonic group, and a phosphate group, or consists of a hardened material of this coating resin.

[Claim 2] The resin board according to claim 1 which is what is chosen from the group which the functional group which has positive charge becomes from the fourth class amino group, a phospho NIUMU machine, a sulfonium machine, a BIGUANIDO machine, and a solid in machine.

[Claim 3] The resin board according to claim 1 which is what is chosen from the group which the functional group which has the third class amino group becomes from a urethane group, a UREA machine, a HIDORAJIDO machine, an amide machine, and an amino group.

[Claim 4] The amino group in living thing material and the functional group which forms a covalent bond An ARUKIRU carbonyl group, The resin board according to claim 1 which is what is chosen from the group which consists of an ARIRU carbonyl group, a formyl group, an epoxy group, an AZURAKUTON machine, an episulphide machine, an acrylyl group, a methacryloyl machine, an acryl amide machine, a methacrylamide machine, and a maleimide machine.

[Claim 5] The resin board according to claim 1 which are at least one or more functional groups chosen from the group which a functional group becomes from the fourth class amino group, a urethane group, a hydroxyl group, an ARUKIRU carbonyl group, an epoxy group, an acrylyl group, and a methacryloyl machine.

[Claim 6] A resin board given in any 1 clause of Claim 1 whose coating resin is acrylic resin to 5.
[Claim 7] A resin board given in any 1 clause of Claim 1 whose coating resin is resin of activity energy line hardenability to 6.
[Claim 8] A resin board given in any 1 clause of Claim 1 which is what is chosen from the group which a resin base material becomes from acrylic resin, styrene system resin, polycarbonate resin, and polyolefin resin to 7.
[Claim 9] The nucleic acid chip characterized by a resin board given in either of 7 coming to fix nucleic acid from Claim 1.
[Claim 10] The gene-analysis method which carries out the hybridization of the nucleic acid and sample nucleic acid which are fixed by this nucleic acid chip using a nucleic acid chip according to claim 9, and is characterized by detecting the intensity of a hybridization.
[Claim 11] The reagent kit for using for a gene-analysis method according to claim 10 characterized by including a nucleic acid chip according to claim 9 at least.
[Claim 12] Are resin for using for fixation of living thing material, and [this resin] Resin for living thing material fixation which has at least one or more functional groups chosen from the group which consists of the functional group which has positive charge, the functional group which has the third class amino group, an amino group and the functional group which can form a covalent bond, a sulfonic group, and a phosphate group, and is characterized by being resin of activity energy line hardenability.

[Detailed Description of the Invention]
[0001]
[Industrial Application] This invention relates to the new resin board which fixes nucleic acid etc. in more detail about the resin board coated with resin in the surface.
[0002]
[Description of the Prior Art] Analysis of the gene by the hybridization method using the substrate which fixed probe nucleic acid is widely conducted, for example in base sequence determination, the gene diagnosis of infection or a hereditary disease, genome gene expression monitoring, etc. In the analysis of these genes, the chip which fixed nucleic acid, such as DNA and RNA, on the substrate is used.
[0003] When it fixes the thing and protein which coated the nitroglycerine cellulose wall, the nylon film, etc. as a substrate which fixes nucleic acid, such as DNA and RNA, for example, poly vinyl FURORIDO (PVDF) is usually used. Moreover, the glass plate which coated JP,2000-63154,A with the surface by the polymer which has a carboxyl group as a functional

group is indicated.
[0004] However, such substrate material also has many which have a fluorescence background high in being unsuitable for formation of minute spot. Furthermore, it was difficult for there to be a problem in the ease of carrying out of the ease of dealing with it, or micro processing etc., and to be highly efficient and to produce a DNA chip in large quantities inexpensive.
[0005]
[Problem(s) to be Solved by the Invention] The technical problem of this invention is fixing nucleic acid, peptide, protein, or an antibody stably efficiently, and offering the suitable resin board for processing of chip-izing etc.
[0006]
[Means for Solving the Problem] This invention persons found out that nucleic acid, such as DNA, was efficiently fixable by coating the surface of a resin board with the resin which has a specific functional group, as a result of repeating research wholeheartedly that the above-mentioned purpose should be attained. Moreover, it finds out that a hybridization reaction can be efficiently carried out in the substrate which fixed the nucleic acid which carried out the sign with the fluorescent substance etc., and that the amount of formation of the above-mentioned hybridization is detectable in high sensitivity by measuring the signal intensity of a sign, and came to complete this invention.
[0007] Namely, the summary of this invention is a resin board used for fixation of living thing material, and [this resin board] The functional group which has positive charge, the functional group which has the third class amino group, the amino group in living thing material and the functional group which forms a covalent bond, It is in the resin board for living thing material fixation characterized by being what a resin base material is coated with the coating resin which has at least one or more functional groups chosen from the group which consists of a hydroxyl group, a sulfonic group, and a phosphate group, or consists of a hardened material of this coating resin.
[0008] Moreover, another summary of this invention is resin for using for fixation of living thing material, and [this resin] It has at least one or more functional groups chosen from the group which consists of the functional group which has positive charge, the functional group which has the third class amino group, an amino group and the functional group which can form a covalent bond, a sulfonic group, and a phosphate group, and is in the resin for living thing material fixation characterized by being resin of activity energy line hardenability.
[0009]
[Embodiment of the Invention] In this invention, nucleic acid, peptide, protein, an antibody, etc. are expressed as "living thing material." A "substrate" fixes living thing material and what consists of a "base material" and "coating resin", the thing which processed coating resin itself

as a substrate, etc. are mentioned.

[0010] The "resin board" of this invention is resin used as a substrate or a base material. Moreover, a deoxyribo nucleotide (DNA) or RIBONUKUREOCHIDO (RNA) is expressed as "nucleic acid." Although the resin board of this invention consists of the coating resin which coats a resin base material and its surface, coating resin itself may be used for it as a base material.

[0011] As a base material, if the technical problem of this invention can be attained, there will be no restriction in particular, but resin, a glass plate, and a silicon plate are mentioned, for example. Also in these, resin excellent in micro-processing nature etc. is desirable. Although not restricted especially as a resin base material, what is excellent in the optical characteristic is desirable. For example, what background noise does not generate at the time of fluorescence detection is desirable.

[0012] As a kind of resin for base materials, the resin constructed for which the bridge or insolubilized based on thermoplastics, thermosetting resin, radical hardenability resin, and solvent meltable precursor resin is mentioned. A single polymer or a copolymer is sufficient, in the case of a copolymer, the arrangement in particular is not limited, for example, a random copolymer, an exchange copolymer, a block copolymer, a graft copolymer, etc. are used.

[0013] In this Description, it writes for a single polymer or a copolymer "acrylic acid (meta)". ["a polymer (**)", acrylic acid, or methacrylic acid] As an example of the above-mentioned resin for base materials (Meta) acrylic resin [, such as a methyl acrylate (**) polymer,]; -- random copolymer [of the copolymer; methyl methacrylate containing polystyrene or it and styrene] (MS resin); -- (Polycarbonate PC); polyalkylene terephthalate; -- [fatty series] Or alicyclic polyamide; Nylon 6T copolymer, the half-aromatic polyamide; amorphous polyamide; poly MECHIRU pen ten of nylon MXD6 grade, Transparency polyolefin, such as a polyethylene (**) polymer or a polypropylene (**) polymer; Polyethylene, Polypropylene Or crystalline polyolefin [, such as poly MECHIRUBUTEN,]; Cyclo OREFIN Or the various kinds guided from Sik Roar Lekain Polymer; (**) hydroxyl group content polymer [, such as polyvinyl alcohol, and vinyl alcohol / alpha olefin copolymer,]; -- polyvinyl acetate; -- poly vinyl pylori boss; -- acrylonitrile styrene resin (AS resin); -- styrene acrylonitrile resin SAN resin; -- Acrylonitrile butadiene styrene resin (ABS resin); chlorine content resin; poly vinyl fluorides, such as polyvinyl chloride and a polyvinylidene chloride, the poly BINIRIDEN fluoride, Fluoro-resins, such as ethylene tetrafluoro ethylene copolymerization resin (ETFE) or poly tetrafluoro ethylene (PTFE); The poly ant rate, Aromatic series system resin which has transparency, such as poly SARUHON and polyether sulphone; Poly ether IMIDO, IMIDO structure content thermoplastics [, such as polyamide IMIDO,]; -- various heat-resistant polyimide; -- poly-phenylene-sulphide; -- poly ether ketone -- Crystalline heat resistance resin, such as poly ether nitril; the rubber and the elastomers etc. which have the resin hardened material; transparency guided from the

compound which has the functional group which has PORIASE tar, TORIASECHIRU cellulose, its partial saponification thing; radical polymerization nature, or thermal polymerization nature are mentioned.

[0014] Preferably Styrene system resin; polycarbonate; polyethylene systems, such as acrylic resin;MS resin, such as a methyl acrylate (meta) (**) polymer, Transparency polyolefin, such as a polypropylene system and alicycle OREFIN; it is thermoplastics, such as various transparency resin guided from the Sik Roar Lekain derivative. As a method of manufacturing such a resin base material, it unites with the characteristic of each resin and the usually performed well-known forming method can be applied. For example, the fabrication depended on notes types, such as injection molding, extrusion molding, compression molding, ejection compression molding, transfer molding, calendar fabrication, and cast fabrication, is mentioned. As the desirable forming method for using this resin base material for the nucleic acid chip which performs delicate detection so much in these There are few forming steps, and since the control technology of forming factors, such as temperature, pressure, speed, and time, is progressing, reproducibility and homogeneity are injection molding, compression molding, etc. from which productivity high at best again is acquired.

[0015] Moreover, although the resin base material obtained by the above-mentioned method is not restricted especially as a method of making it into desired form, the method of carving with edged tools, such as a cutter and the Thompson edge, the method of using a desired metallic mold at the time of fabrication, etc. are mentioned, for example. As the form and the size of a resin base material, the 0.2-2-mm-thick range and a size have the desirable rectangle whose one side is 10-200mm, and its form of the slide glass (75mmx25mmx1mm) usually used is more desirable. As form of the resin board for nucleic acid fixation, when using as a DNA chip, known is usually used in itself, for example, be [easy but / it] it is also possible to process it so that it may have a slot for circulating a sample or holding.

[0016] As coating resin used for this invention, living thing material, for example, nucleic acid, amino acid, peptide, protein, or an antibody is resin with an efficiently combinable functional group. As a bonding pattern, especially if coating resin and living thing material can join together efficiently, it will not be restricted, but an ionic bond, a covalent bond, a hydrogen bond, etc. are mentioned, for example. In these, the combined use with a covalent bond or a hydrogen bond is desirable including an ionic bond with [resin / coating / positive charge] a minus electric charge in living thing material. Moreover, when coating resin and living thing material join together by a covalent bond, existence of the positive charge in coating resin is effective in preventing nonspecific adsorption of living thing material.

[0017] As a desirable functional group of coating resin For example, the fourth class amino group (the fourth class ammonium machine), a phospho NIUMU machine, a sulfonium machine, What has positive charge, such as a BIGUANIDO machine and a solid in machine; A

urethane group, a UREA machine, What has the third class amino groups, such as a HIDORAJIDO machine, an amide machine, and an amino group; An ARUKIRU carbonyl group, An ARIRU carbonyl group, a formyl group, an epoxy group, an AZURAKUTON machine, Amino groups, such as an episulphide machine, an acrylyl group, a methacryloyl machine, an acryl amide machine, a methacrylamide machine, and a maleimide machine, and the thing; hydroxyl group; sulfonic group which forms a covalent bond; a phosphate group etc. is mentioned. Although these functional groups may be made to exist independently in coating resin, it is desirable to make it combine and exist.

[0018] The fourth class amino group, a urethane group, a hydroxyl group, an ARUKIRU carbonyl group, an epoxy group, an acrylyl group, and a methacryloyl machine are more desirable among the above-mentioned functional group. Furthermore, it is making it exist combining these functional groups two or more sorts preferably. For example, the fourth class amino group, a urethane group and the fourth class amino group, a hydroxyl group and the fourth class amino group, an acrylyl group, etc. are mentioned. Furthermore, the combination of the combination of the fourth class amino group, a urethane group, and a hydroxyl group and the fourth class amino group, a urethane group, and an acrylyl group etc. is mentioned preferably. Coating resin containing the fourth class amino group is excellent also in electrification tightness.

[0019] Especially as a method of making coating resin containing the above-mentioned functional group, it is not restricted but the method explained to a well-known method or the well-known following can be applied. The method which coating resin is made to contain especially about a desirable thing also in the above-mentioned functional group is illustrated below. For example, the surface-active agent which has the fourth (1-A) class amino group is made to contain in a KOTEINGU agent as a method of introducing the fourth class amino group. The radical polymerization nature monomer which holds the method made [the KOTEINGU film surface] to carry out BURIDO out and the fourth (1-B) class amino group is made to contain in a KOTEINGU agent. The method of carrying out activity energy line irradiation and fixing on the surface and the polymer which has the fourth (1-C) class amino group are made to contain in a KOTEINGU agent. Carry out activity energy line irradiation if needed, make the Silang coupling agent which has the method or (1-D) the fourth class amino group fixed on the surface contain, it is made to react with other KOTEINGU ingredients, and the method of fixing the fourth class amino group on the surface etc. is mentioned.

[0020] In these, a concrete method [being desirable (1-C)] is explained in full detail below. The fourth class of acrylyl group content amino compound 10 - 90 weight part, (1-C-1) (meta) Acrylic ester 90 - 10 weight part and if needed Ester or amide of hydroxyalkyl machine content (meta) acrylic acid, (Meta) The polymerization nature monomer mixture which carried out 1-20 weight part combination of the ester of ARUKIRU carbonyl group content (meta) acrylic acid or

amide, and the formyl group content polymerization nature monomer, and was made into a total of 100 weight parts is uniformly dissolved in hydrophilic solvents, such as a water solvent or lower alcohol. It is obtained by adding radical polymerization initiators, such as organic peroxide or an azo compound, 0.3 to 2% of the weight, and carrying out copolymerization preferably, 0.1 to 10% of the weight. Here, you may carry out copolymerization by the well-known method used for polymerizations of acrylic ester, such as a polymerization or anionic polymerization (meta) which used a living radical polymerization and complex catalyst other than a radical polymerization.

[0021] (Meta) As an example of the fourth class of acrylyl group content amino compound, beta-(meta) AKURIROIRU ethyl trimethylammonium chloride, beta-(meta) AKURIROIRU ethyl triethyl ammonium chloride, etc. are mentioned. (Meta) as an example of acrylic ester MECHIRU (meta) acrylate, butyl (meta) acrylate, 2-ethylhexyl (meta) acrylate, The straight chain of the carbon numbers 1-20, such as lauryl (meta) acrylate and stearyl (meta) acrylate, or ARUKIRU (meta) acrylic ester of branched chain; Cyclohexyl (meta) acrylate, Isobornyl (meta) acrylate, adamanthyl (meta) acrylate, Tricyclodecane methanol (Meta) cycloalkyl (meta) acrylic ester [of the carbon numbers 3-20, such as acrylic ester,]; -- Silang content (meta) acrylic ester [, such as trimethoxysilylpropyl (meta) acrylate,]; -- [trifluoro ethyl (meta) acrylate etc.] Full OROARUKIRU machine content (meta) acrylic ester; (Par) Saturation annular machine content (meta) acrylic ester, such as tetrahydrofurfuryl (meta) acrylate, etc. can be mentioned. In addition, as long as it is the quantity which hardly shows absorption to a wavelength field of 400nm or more, aromatic ring content (meta) acrylic ester, such as Ben Jill (meta) acrylate and FENOKISHI ethyl (meta) acrylate, etc. may also be included.

[0022] As the ester of hydroxyalkyl machine content (meta) acrylic acid, or an example of amide 2-hydroxyethyl (meta) acrylate, 2-hydroxyethyl (meta) acryl amide, 4-hydroxy butyl (meta) acrylate, 4-hydroxy butyl (meta) acryl amide, KAPURO lactone denaturation 2-hydroxyethyl (meta) acrylate, etc. can be mentioned.

[0023] As the ester of ARUKIRU carbonyl group content (meta) acrylic acid, and an example of amide, beta-aceto ethyl (meta) acrylate, diacetone (meta) acryl amide, etc. can be mentioned. AKUROREIN, meta-KUROREIN, etc. can be mentioned as an example of a formyl group content polymerization nature monomer.

[0024] The ester or the amide of the third class amino group content (meta) acrylic acid (1-C-2) 10 - 90 weight part, Acrylic ester 90 - 10 weight part and if needed Ester or amide of hydroxyalkyl machine content (meta) acrylic acid, (Meta) It dissolves in an organic solvent uniformly and the copolymerization of the polymerization nature monomer mixture which carried out 1-20 weight part combination of the ester of ARUKIRU carbonyl group content (meta) acrylic acid or amide, and the formyl group content polymerization nature monomer, and was made into a total of 100 weight parts is carried out to it like the above. It is obtained

by making the obtained copolymer react for several hours at alkylating agents, such as alkyl halide, JIARUKIRU sulfuric acid, Alekan alkyl sulfonate ester, HAROKARUBON acid (ester), halogenation ARUKENIRU, or halogenation cycloalkyl, and 40-100 degrees C.

[0025] As the ester of the third class amino group content (meta) acrylic acid, or an example of amide, acrylic acid (meta) dimethylaminoethyl, dimethylaminoethyl (meta) acryl amide, acrylic acid (meta) dimethylamino butyl, etc. are mentioned. (Meta) The ester of acrylic ester, the ester of hydroxyalkyl machine content (meta) acrylic acid or amide, and ARUKIRU carbonyl group content (meta) acrylic acid or amide, and a formyl group content polymerization nature monomer are synonymous with the above.

[0026] As an example of alkyl halide, chlorination MECHIRU, a methyl bromide, iodination MECHIRU, chlorination butyl, etc. are mentioned. JIMECHIRU sulfuric acid, diethyl sulfate, etc. are mentioned as an example of JIARUKIRU sulfuric acid. As an example of Alekan alkyl sulfonate ester, methanesulfonic acid MECHIRU, methanesulfonic acid ethyl, etc. are mentioned. As an example of HAROKARUBON acid (ester), chloracetic acid, sodium chloroacetate, chloro ethyl acetate, methyl chloroacetate, 3-chloro methyl propionate, etc. are mentioned. As an example of halogenation ARUKENIRU, an allyl chloride, chlorination metallyl, chlorination Cros Chill, bromination ARIRU, etc. are mentioned. Chlorination cyclohexyl, iodination cyclopentyl, etc. are mentioned as an example of halogenation cycloalkyl.

[0027] The ester or the amide of the third class amino group content (meta) acrylic acid (1-C-3) 10 - 90 weight part, The ester or the amide of 90 - 10 weight part and hydroxyalkyl machine content (meta) acrylic acid for acrylic ester 1 - 20 weight part, (Meta) The copolymerization of the polymerization nature monomer mixture which carried out 1-20 weight part combination of the ester of ARUKIRU carbonyl group content (meta) acrylic acid or amide, and the formyl group content polymerization nature monomer, and was made into a total of 100 weight parts is carried out by the same method as the above if needed. The inside of the solvent which does not contain isocyanate groups, such as a ketone solvent and an aromatic hydrocarbon solvent, and the functional group which has reactivity under existence of organic tin compound catalysts, such as dibutyltin dilaurate, An acrylyl group (meta) content iso cyanate compound is made to react to the hydroxyl group of this copolymer in 20-100 degrees C and several hours. The copolymer containing the fourth class amino group, a hydroxyl group, an acrylyl group or a methacryloyl machine, and a urethane group is obtained by transforming the third class amino group for this to the fourth class amino group by an alkylating agent like the above.

[0028] (Meta) as an acrylyl group content iso cyanate compound, for example, 2-methacryloyl ethyl iso cyanate, 2-AKURIROIRU ethyl iso cyanate, etc. should mention, and be -- **. Moreover, hydroxyl group content mono-** JI or Pori (meta) acrylate, such as hydroxyethyl acrylate, TORIMECHI roll propane diacrylate, and a PENTA erythritol TORIAKURI rate, The

compound which left the isocyanate group may be used by a reaction thing with the iso cyanate compound of two organic functions, such as isophorone diisocyanate, hexamethylene di-isocyanate, and its trimer, to many organic functions.

[0029] The ester or the amide of the third class amino group content (meta) acrylic acid (1-C-4) 10 - 90 weight part, 90 - 10 weight part and epoxy group content (meta) acrylate for acrylic ester 1 - 20 weight part, (Meta) If needed Ester or amide of hydroxyalkyl machine content (meta) acrylic acid, The copolymerization of the polymerization nature monomer mixture which carried out 1-20 weight part combination of the ester of ARUKIRU carbonyl group content (meta) acrylic acid or amide, and the formyl group content polymerization nature monomer, and was made into a total of 100 weight parts is carried out like the above. A basic catalyst or TORIFENIRUHOSU fins, such as triethyl amine and dimethylamino PIRIJIN, Acrylic acid (meta) and an epoxy group are made to react under acidity, such as tributyl phosphite, - existence of a neutral catalyst in 50-150 degrees C and several hours - tens of hours among the solvent which does not contain epoxy groups, such as a ketone solvent and an aromatic hydrocarbon solvent, and the functional group which reacts easily. The copolymer containing the fourth class amino group, a hydroxyl group, an acrylyl group or a methacryloyl machine, and a urethane group is obtained by changing the third class amino group into the fourth class amino group for this by an alkylating agent like the above.

[0030] As an example of epoxy group content (meta) acrylate, glycidyl (meta) acrylate, the acrylate which has a cyclo HEKISENOKISHIDO frame (meta), etc. can be mentioned. For example, made the compound which has the isocyanate group of two (2-A) or more organic functions, and the compound which has the hydroxyl group of two or more organic functions react beforehand as the introductory method of a urethane group. How to make the urethane (meta) acrylate which introduced the acrylyl group or the methacryloyl machine into oligo - polyurethane, or this, and made it hold activity energy line hardenability contain, (2-B) Make the compound which has an isocyanate group react to the polymer containing a hydroxyl group. The compound which has a hydroxyl group is made to react to the polymer containing the method or (2-C) isocyanate group which makes a side chain contain the polymer which has a urethane group and a hydroxyl group, and the method of making the polymer which has a urethane group and a hydroxyl group to a side chain contain etc. is mentioned.

[0031] As urethane (meta) acrylate, an iso cyanate compound, the addition of hydroxyl group content (meta) acrylate, or the addition of isocyanate group content (meta) acrylate and various hydroxyl group-containing compound can be mentioned, for example. As a urethane group content (**) polymer, for example A straight chain or the solvent soluble polyurethane of partial branching, The compound to which the polymer which uses the ester or the amide of hydroxyalkyl machine content (meta) acrylic acid as an essential ingredient (**) was made to add iso cyanate, The polymerization thing of the Pori hydroxyl compound and a PORIISO

cyanate compound, and epoxy group content (meta) acrylate to the polymer used as an essential ingredient (**) After carboxylic acid addition, The compound which made iso cyanate add to a generation hydroxyl group, the compound which made the hydroxyl group add to the polymer which uses isocyanate group content (meta) acrylate as an essential ingredient (**), etc. can be mentioned.

[0032] As the introductory method of a hydroxyl group, the method of making a hydroxyl group (2-D) content (**) polymer containing, the method of making hydroxyl group (2-E) content (meta) acrylate contain, etc. are mentioned, for example. As hydroxyl group content (meta) acrylate, besides the above, for example Hydroxyl group content di(meth)acrylate, such as TORIMECHI roll propane diacrylate, The acrylic acid (meta) addition of epoxy (meta) acrylate, such as an acrylic acid (meta) addition to hydroxyl group content polyfunctional (meta) acrylate, such as a PENTA erythritol TORIAKURI rate, and glycidyl (meta) acrylate, etc. can be mentioned.

[0033] As a hydroxyl group content (**) polymer, for example The polymer which used the ester or the amide of hydroxyalkyl machine content (meta) acrylic acid as the essential ingredient (**); Epoxy group content (meta) acrylate is used as an essential ingredient. The acrylic acid (meta) accretionary prism to the polymer included (**) Or ******; by the water of an epoxy group polyvinyl alcohol; -- vinyl alcohol machine content copolymer [, such as ethylene / vinyl alcohol copolymer,]; -- partial saponification thing [of an acetic acid vinyl (**) polymer]; -- TORIASECHIRU cellulose or its partial saponification thing; -- FENOKISHI resin; -- [hydroxymethyl styrene etc. / hydroxyl group content styrene] Polymer; (**) The transparency polyolefin which carried out the copolymerization of hydroxyl group content OREFIN can be mentioned.

[0034] How to, make a formyl group (3-A) or an ARUKIRU carbonyl group content compound contain in KOTEINGU resin as a method of introducing a carbonyl group for example, (3-B) How to oxidize and to change a surface hydroxyl group into a formyl group or an ARUKIRU carbonyl group after applying the KOTEINGU resin which has a hydroxyl group, (3-C) After applying KOTEINGU resin containing an alkyloxy carbonyl group, After applying KOTEINGU resin containing the carbonyl group protected in the form of the method of carrying out partial reduction of the surface alkyloxy carbonyl group, and changing into a formyl group or an ARUKIRU carbonyl group, ASETARU, or KETARU, Acid treatment of the surface is carried out and the method of ******(ing) ASETARU or a KETARU machine etc. is mentioned. The method of (3-A) is desirable in these.

[0035] [a formyl group or an ARUKIRU carbonyl group content (**) polymer] For example, the polymer which contains the ester of the above-mentioned ARUKIRU carbonyl group content (meta) acrylic acid or amide, and/or a formyl group content polymerization nature monomer as an essential ingredient (**), Pori ketone, such as an alternating copolymer of OREFIN and

carbon monoxide, a carbonyl group content (meta) acrylate (**) polymer, etc. are mentioned.
[0036] (Meta) The method of, for example, making the radical polymerization nature compound which has an acrylyl group (meta) contain in the state of a monomer or a polymer as the introductory method of an acrylyl group is mentioned. (Meta) as an acrylate machine content polymer The polymer which made isocyanate group content (meta) acrylate add to the polymer which contains the ester or the amide of monofunctional or polyfunctional (meta) acrylate, and the above-mentioned hydroxyalkyl machine content (meta) acrylic acid as an essential ingredient (**), The polymer which made acrylic acid (meta) add to the polymer which contains the above-mentioned epoxy group content (meta) acrylate as an essential ingredient (**) is mentioned.
[0037] As the introductory method of an epoxy group, the method of making the radical polymerization nature monomer containing the radical polymerization nature polymer or epoxy group which has an epoxy group etc. contain is mentioned to a side chain, for example. As an epoxy group content polymer, the polymer which includes OREFIN structure as an essential ingredient (**) is oxidized, for example, and a polymer (**), epoxy (meta) acrylate, an epoxy resin, etc. which changed the ARUKENIRU machine into the epoxy group are mentioned.
[0038] The method and functional group which were explained above are a thing for illustration, and should understand not being limited to these. The KOTEINGU resin composition needs to contain preferably the ingredient containing at least one sort in the above-mentioned functional group 5% of the weight or more 1% of the weight or more.
[whether these ingredients are Polymer Division-like or it has an activity energy line hardenability functional group] When the KOTEINGU film after an application or hardening has the hardness more than H with the intensity more than fixed, or the pencil hardness at the time of a Pori methyl methacrylate application, you may form KOTEINGU resin by these ingredient independent.
[0039] However, the compound which has the above-mentioned functional group is a monomer or a low polymer. And the case where the KOTEINGU film after an application or hardening does not have sufficient intensity and hardness, and when you need higher intensity and hardness, you may make it contain the KOTEINGU resinous principle except having described above to raise functional group concentration. The polymer which is excellent in compatibility with the ingredient of high hardness, such as Pori methyl methacrylate (PMMA), and others although not restricted especially as this example; A PENTA erythritol TORIAKURI rate, Dipentaerythritol pentaacrylate, dipentaerythritol hexaacrylate, Acrylate of three or more organic functions, such as trimethylolpropane triacrylate and ditrimethylolpropanetetraacrylate (meta); Cyclohexyl methacrylate, One to 2 organic functions, such as cyclohexane dimethanol diacrylate, (Meta) acrylate; -- urethane acrylate; -- polyester acrylate; -- light, such as epoxy resins, such as a cyclo HEKISENOKISHIDO frame content epoxy resin, or thermosetting resin;

-- thermosetting resin [, such as maleimide resin and oxazoline resin,]; -- silica -- silica -- inorganic filler [, such as sol,]; -- the inorganic filler; vinyl group which introduced the unsaturated bond which can be hardened with an inorganic filler precursor; light or heat, such as a reaction thing of silicate, silicate / titanate, a methacryloyl machine, an epoxy group, an amino group, a mercapto group, and an isocyanate group, Functional group content Silang coupling agents, such as the fourth class amino group; the Silang coupling agent which has ARUKIRU machines, such as ARUKIRUTORI alkoxy silyl SHIRAN, can be mentioned.

[0040] The coating resin manufactured by the above-mentioned method is diluted with a solvent etc., and a substrate is manufactured by making a film form on a base material. As the formation method of the KOTEINGU film to a base material top To a transparency resin base material, for example, the DEIPPU coat method, a spin coat method, the flow coat method, [with the **** method with **** instruments, such as a spray coating method, the bar coat method and a photogravure coat, a roll coat, a braid coat, and an air knife coat,] Activity energy line irradiation is carried out solvent dryness and if needed, and the method of ****(ing) 0.1-50 micrometers, so that a 0.2-5-micrometer smooth KOTEINGU film may be obtained preferably is mentioned to the base material surface.

[0041] In order to attain the diameter of spot with suitable nucleic acid solution etc. as an angle of contact over the water of the generated KOTEINGU film, 40 degrees or more are desirable, and in order to make nucleic acid etc. fully fix, 90 or less degrees is desirable. Therefore, the range with a desirable angle of contact to water is 50 to 80 degrees still more preferably 40 to 90 degrees. The Hayes value estimates the transparency of the generated KOTEINGU film. In this case, it is less than +1% preferably, and needs to be [less than +2% of Hayes value of a base material] uniform.

[0042] The hardness of the generated KOTEINGU film of more than the pencil hardness H is more than 2H preferably. When using it in the environment where especially a handling top crack is attached easily, it is using it combining activity energy line hardenability polyfunctional (meta) acrylate, urethane acrylate or an inorganic filler, an inorganic filler precursor, etc., and it is desirable to set up more than pencil hardness 4H.

[0043] In order to adjust the physical properties of the generated KOTEINGU film to the above-mentioned range, or in order to give the function of others, such as improvement of coat physical properties Ultraviolet ray absorbent; Either of the additive agents blended with light stabilizer; antiblocking agent; slip additive; leveling agent; antifoaming agent; support prevention agents, such as antioxidant; hindered amine systems, such as a hindered phenol system, a sulfur system, or the Lynn system, etc. during coat composition It may be made to blend 0.01 to 2% of the weight, respectively.

[0044] Moreover, in order to adjust the viscosity of a coat constituent, the same thing as the solvent used at the time of polymer manufacture can be used. When irradiating an activity

energy line, as this activity energy line and the irradiation method Although not limited in particular, as this activity energy line for example, the ultraviolet rays emitted from light sources, such as a xenon lamp, a low-pressure mercury lamp, a high-pressure mercury lamp, a very-high-pressure mercury lamp, a metal halide lamp, carbon arc light, or a tungsten lamp, -- or Usually, activity energy lines taken out from a 20-2000kV electron beam accelerator, such as an electron beam, alpha line, a beta ray, or a gamma ray, are mentioned.

[0045] When activity energy lines are ultraviolet rays, it usually carries out using a photo polymerization initiator. As a photo polymerization initiator, benzoin methyl ether, benzoin ethyl ether, Benzoin iso-propyl ether, benzoin isobutyl ether, Diethoxy aceto FENON, benzoRUJIMECHIRUKE tar, 2-hydroxy 2-methylpropiohenone, 1-hydroxy cyclohexyl phenyl ketone, benzoFENON, 2, 4, 6-TORIMECHIRUBENZO in diphenyl phosphine oxide, 2-******-[4-(MECHIRUCHIO) FENIRU]-2-morpholino 1-pro PANON, 2-benzoroux 2-dimethylamino 1 -(4-morpholino FENIRU)- Butane 1-ON, MIHIRAZU ketone, N, and N-dimethylamino benzoic acid iso AMIRU, 2-chloro thioxan ton, 2, and 4-JIECHIRU thioxan ton etc. is mentioned, and these photo polymerization initiators can also use two or more sorts together suitably.

[0046] the amount of the photo polymerization initiator used -- 1- of the sum of a KOTEINGU resinous principle -- it is 1 to 5% of the weight of a range preferably 10% of the weight. As a wavelength of ultraviolet rays, 200-400nm and irradiation time are usually 0.1 to 60 seconds. The coating resin hardened by activity energy line irradiation is excellent in abrasion resistance, transparency, and solvent resistance.

[0047] By the above-mentioned method, coating resin and the coated resin board of this invention are obtained. This is suitable for fixation of living thing material, such as nucleic acid, peptide, protein, and an antibody. It is suitable for nucleic acid fixation of DNA etc. in particular. Furthermore, it is a substrate suitable also for micro processing, such as chip-izing and formation of a micro array. Therefore, the new coating resin board of this invention can be effectively used as a substrate of a nucleic acid chip, and can analyze sample nucleic acid efficiently. Moreover, this invention enables it to produce a highly efficient nucleic acid chip in large quantities inexpensive.

[0048] "Sample nucleic acid" means an oligonucleotide and the nucleic acid made to hybridize for the purpose of the analysis of a base sequence here. When analyzing sample nucleic acid by a hybridization, it is desirable that the sign of either sample nucleic acid or an oligonucleotide is carried out. The method in particular of sign-izing is not limited and can mention the technique using [for example,] radioisotope and a fluorescence pigment etc. The result of a hybridization can be measured by the method adapted to various sign methods. However, in the gene expression monitoring method, since the strength of discovery between sample nucleic acid is detectable in parallel by carrying out the sign of the sample nucleic acid with several fluorescence pigments with which detection wavelengths differ, the sign by a

fluorescence method is used preferably.
[0049] It is applicable to diagnosis of the determination of the DNA base sequence using the hybridization method as an example of application of base sequence analysis, infection, and a genetically determined disease, mapping of the huge genome DNA, gene expression monitoring, etc. As a diagnostic method of infection, DNA is extracted from a subject's blood etc., a DNA probe is produced from arrangement peculiar to various pathogenic organs by the method of this invention to the DNA, for example, a hybridization reaction is performed, and the method of detecting existence of a pathogenic organ is mentioned. As a diagnostic method of a genetically determined disease, an oligonucleotide is produced by the method of this invention based on arrangement specific in the cause gene of a hereditary disease, a hybridization with the chromosomal DNA obtained from the subject is performed, and the existence of the variation in the gene is detected. Although mapping of the huge genome DNA is technology indispensable in a genome DNA analysis project, the arrangement on the genome of each clone can be determined by performing the DNA probe and hybridization of a large number produced by the method of this invention to the genome bank.
[0050] Moreover, the DNA chip manufactured using the new coating resin board of this invention has many amounts of DNA fixation, since it is stable, when conducting a gene analysis by the hybridization method, is highly sensitive and can be quantitatively used with sufficient reproducibility. Method [A. which compounds a direct oligonucleotide on a substrate as the manufacture method of a DNA chip C.Pease et al., Proc.Natl.Acad.Aci.USA, 91, and 5022-5026] (1994), The compound oligonucleotide [Z.Guo et al., Nucl.Acids Res., 22, and 5456-5465] (1994), Or [M., such as a PCR product, The method of fixing Schena et al., Science, 270, and 467-470] (1995) on a glass substrate is mentioned. Especially the resin board of this invention is effective in the latter.
[0051] Moreover, in this gene-analysis method, the well-known thing usually used is applied and it deals in reagent kits, such as cleaning fluid, a sample diluted solution, and hybridization solution.
[0052]
[Example] Although a synthetic example and an example are given and this invention is explained still in detail hereafter, this invention is not limited to these synthetic examples and examples. In addition, the part and % which are indicated in a synthetic example and the example mean weight part and weight %, respectively. In addition, about evaluation of the basic physical properties of a KOTEINGU film, it carried out by the following method.
[0053] Transparency (Hayes value %): JIS K It measured based on 7105.
Surface-resistance value: It measured as a value for impressed-electromotive-force 100 V or 1 minute using the surface resistance meter (the Takeda Riken make: TR8601 type) using the sample which carried out state adjustment for 24 hours or more in 23 degrees and 65%

relative humidity.
Pencil hardness: JIS K It measured based on 5400.
[0054] Angle of contact: Using the Kyowa Chemistry nature P type angle-of-contact measuring instrument, 23 degrees, using the sample which carried out state adjustment in 65% of relative humidity for 24 hours or more, water or 0.05%DNA (3XSSC solutions) solution was dropped at the 0.002ml measurement surface, and it measured at 23 degrees.
Adhesion nature (cross cut test): JIS K It measured based on 5400.
[0055] The mixture of 60 copies of 30 copies of synthetic example 1 cyclohexyl methacrylate, ten copies of 2-hydroxyethyl methacrylate and N, and N-dimethylaminoethyl methacrylate and 200 copies of methyl ethyl ketone is heated, When it ****(ed) at 65 degrees C, 0.6 copy of 2 and 2'-azobis (2,4-dimethylvaleronitrile) was added 2 hours afterward from the time of **** at each 65 degrees C, respectively, it reacted at 80 degrees C at 65 degrees C for 2 hours for 5 hours, and the copolymer solution (P-1) of 33% of solid content was obtained. To this thing [57.1 copies of mixtures (OSAKA ORGANIC CHEMICAL INDUSTRY /, LTD. / make: the screw coat 300, hydroxyl value 131 mgKOH/g) of 22.2 copies of isophorone diisocyanate, a PENTA erythritol TORIAKURI rate, and PENTA erythritol tetraacrylate] The reaction was ended, after having added 79.3 copies of additions obtained by having made react for 8 hours at 25 degrees C - 80 degrees C, reacting at 80 degrees for 5 hours and checking disappearance of absorption of the isocyanate group of 2250cm-1 with an infrared absorption spectrum. As a result, the copolymer solution (P-2) of 45% of the solid content which has an acrylyl group to a side chain was obtained.
[0056] Next, after diluting the obtained copolymer solution (P-2) so that it may become 20% of solid content with isopropyl alcohol, chlorination MECHIRU was introduced into the system of reaction, it reacted at 50 degrees C for 6 hours, and the copolymer solution (III-1) of 25% of the solid content which has the fourth class amino group, a urethane group, and an acrylyl group was obtained.
The mixture of 70 copies of synthetic example 2 methyl methacrylate, 30 copies of 2-hydroxyethyl methacrylate, and 200 copies of methyl ethyl ketone is heated, When it ****(ed) at 65 degrees C, 0.6 copy of 2 and 2'-azobis (2,4-dimethylvaleronitrile) was added 2 hours afterward from the time of **** at each 65 degrees C, respectively, it reacted at 80 degrees C at 65 degrees C for 2 hours for 5 hours, and the radical copolymer solution (P-3) which has the hydroxyl group of 33% of solid content was obtained.
[0057] The mixture of 85 copies of synthetic example 3 methyl methacrylate, 15 copies of diacetone acrylamide, and 200 copies of methyl ethyl ketone is heated, When it **** at 65 degrees C, from the time of ****, in 2 hours, add 0.6 copy of 2 and 2'-azobis (2,4-dimethylvaleronitrile) at each 65 degrees C, respectively, and it reacts to them at 80 degrees C at 65 degrees C for 2 hours for 5 hours. The radical copolymer solution (P-4) of 33% of the

solid content which has a MECHIRU carbonyl group was obtained.
[0058] The mixture of 85 copies of synthetic example 4 methyl methacrylate, 15 copies of glycidyl methacrylate, and 200 copies of methyl ethyl ketone is heated, When it ****(ed) at 65 degrees C, 0.6 copy of 2 and 2'-azobis (2,4-dimethylvaleronitrile) was added 2 hours afterward from the time of **** at each 65 degrees C, respectively, it reacted at 80 degrees C at 65 degrees C for 2 hours for 5 hours, and the radical copolymer solution (P-5) of 33% of the solid content which has an epoxy group was obtained.
[0059] Example 1 copolymer solution (III-1) The mixture (Nippon Kayaku: Kaya Rudd DPHA) of ten copies, dipentaerythritol hexaacrylate, and dipentaerythritol pentaacrylate 90 copies, The IRUGA cure 184 was diluted with three copies, two copies were diluted with the mixed solvent of iso propanol and methyl ethyl ketone for triethanol amine, and the KOTEINGU resin solution of 35% of solid content was prepared. this KOTEINGU resin solution is further diluted with iso propanol to 2.5 to 20% of solid content (viscosity 2 - 5 mPa-s (25 degrees C)), and the film thickness after dryness is set to about 2-5 micrometers -- as After applying to the PMMA board or PC board into which 1mm in thickness and the size of 75mm x 25mm were processed by bar KOTA, it dried for 2 minutes at 60 degrees C. In addition, as environment at the time of an application, an application and dryness Eria were 20-25 degrees C in temperature at the time of 1000 and an application the degree about 10000-100000 of cleanness, and directly under the filter, and were 20 to 50% in relative humidity. This board was fixed to the position of 15cm under the light source of the high-pressure mercury lamp of power density 120 w/cm, and the light source, ultraviolet rays were irradiated by 300 mJ/cm2, and the KOTEINGU film was formed.
[0060] KOTEINGU resin solution prepared in the example 2 example 1 It diluted with iso propanol to 2.5% of solid content (viscosity 2 mPa-s (25 degrees C)), and after pulling up to both sides of the PMMA board or PC board which processed 1mm in thickness, and the size of 75mm x 25mm by DEIPPUKOTA and applying to them by speed 200 mm/min, it dried for 2 minutes at 60 degrees C. This board was fixed to the position of 15cm under the light source of the high-pressure mercury lamp of power density 120 w/cm, and the light source, ultraviolet rays were irradiated by 300 mJ/cm2 at both sides, and the KOTEINGU film was formed in both sides.
[0061] Example 3 copolymer solution (P-3) The mixture (Nippon Kayaku: Kaya Rudd DPHA) of 50 copies, dipentaerythritol hexaacrylate, and dipentaerythritol pentaacrylate 30 copies, 20 copies and the IRUGA cure 184 were diluted with one copy, one copy was diluted [the urethane acrylate to which isophorone diisocyanate and a PENTA erythritol TORIAKURI rate were made to react] with methyl ethyl ketone for triethanol amine, and the KOTEINGU resin solution of 35% of solid content was prepared. Further, it diluted with methyl ethyl ketone to 15% of solid content (viscosity 5 mPa-s (25 degrees C)), and after applying this KOTEINGU

resin solution to a PMMA board or PC board by bar KOTA so that the film thickness after dryness may be set to about 2-5 micrometers, it was dried for 2 minutes at 60 degrees C. This board was fixed to the position of 15cm under the light source of the high-pressure mercury lamp of power density 120 w/cm, and the light source, ultraviolet rays were irradiated by 300 mJ/cm2, and the KOTEINGU film was formed.

[0062] The KOTEINGU resin solution prepared in the example 4 example 3 was diluted with methyl ethyl ketone to 8% of solid content (viscosity 4 mPa-s (25 degrees C)), and after pulling up by DEIPPUKOTA and applying to both sides of a PMMA board or PC board by speed 400 mm/min, it dried for 2 minutes at 60 degrees C. This board was fixed to the position of 15cm under the light source of the high-pressure mercury lamp of power density 120 w/cm, and the light source, ultraviolet rays were irradiated by 300 mJ/cm2 at both sides, and the KOTEINGU film was formed in both sides.

[0063] Example 5 copolymer solution (P-4) was further diluted with methyl ethyl ketone to 15% (viscosity 4 mPa-s (25 degrees C)) of solid content, after applying to a PMMA board by bar KOTA so that the film thickness after dryness may be set to about 0.5-2 micrometers, it dried for 2 minutes at 60 degrees C, and the KOTEINGU film was created.

[0064] After having diluted with methyl ethyl ketone the KOTEINGU resin solution prepared in the example 6 example 5 to 8% (viscosity 4 mPa-s (25 degrees C)) of solid content, pulling up it by DEIPPUKOTA and applying it to PMMA board both sides by speed 400 mm/min, it dried for 2 minutes at 60 degrees C, and the KOTEINGU film was created.

[0065] After having diluted further example 7 copolymer solution (P-3) with methyl ethyl ketone to 8% (viscosity 4 mPa-s (25 degrees C)) of solid content, pulling up by DEIPPUKOTA and applying to PMMA board both sides by speed 400 mm/min, it dried for 2 minutes at 60 degrees C, and the KOTEINGU film was created.

[0066] After having diluted further example 8 copolymer solution (P-5) with methyl ethyl ketone to 8% (viscosity 4 mPa-s (25 degrees C)) of solid content, pulling up by DEIPPUKOTA and applying to PMMA board both sides by speed 400 mm/min, it dried for 2 minutes at 60 degrees C, and the KOTEINGU film was created.

[0067] The result of having evaluated the basic physical properties of the KOTEINGU film created in the example 1-8 is shown in Table 1.

[0068]

[Table 1]

表 1

実施例	基材	ヘイズ値	表面抵抗値	鉛筆硬度	接触角（水、DNA）	密着性
1－1	PMMA	0.6	$1.1x10^{11}$	5H	53, 55	100/100
1－2	PMMA	0.7	$9.4x10^{12}$	5H	67, 67	100/100
1－3	PC	0.9	$3.0x10^{13}$	3H	66, 62	100/100
2－1	PMMA	0.2	$2.1x10^{12}$	5H	69, 66	100/100
2－2	PC	0.6	$5.1x10^{12}$	3H	62, 61	100/100
3－1	PMMA	0.3	$>1x10^{16}$	4H	64, 61	100/100
3－2	PC	0.7	$>1x10^{16}$	2H	60, 57	100/100
4－1	PMMA	0.5	$>1x10^{16}$	4H	66, 62	100/100
4－2	PC	0.8	$>1x10^{16}$	2H	62, 60	100/100
5－1	PMMA	0.4	$>1x10^{16}$	2H	64, 63	100/100
6－1	PMMA	0.4	$>1x10^{16}$	2H	65, 65	100/100
7－1	PMMA	0.2	$>1x10^{16}$	H	70, 63	100/100
8－1	PMMA	0.2	$>1x10^{16}$	H	62, 66	100/100

[0069] Notes: the Hayes value of PMMA = manufacture of 0.2 and Hayes value =0.5 example 9 probe of PC was performed by the PCR method as follows. The housekeeping gene of Clontech RT-PCR Control Amplimer Sets was used for the gene used for probe manufacture.
[0070] The list of seven sorts of genes used for probe manufacture is described below.
[0071]
[Table 2]

表 2

遺伝子名	[GenBank accession #]	発現量
(1)beta-Actin	[X00351]	High
(2)Glyceraldehyde-3-Phosphate Dehydrogenase (G3PDH)	[X01677]	High
(3)23-kDa Highly Basic Protein	[X56932]	Medium
(4)alpha-Tublin	[K00558]	High
(5)Transferrin Receptor	[AF187320]	Medium
(6)Hypoxanthine Phosphoribosyltransferase (HPRT)	[V00530]	Medium
(7)Ribosomal protein S9	[U14971]	Medium

[0072] The arrangement of the primer DNA for PCR used for amplification of each gene and

the size of an amplification fragment are as follows.

[0073]

[Table 3]

表 3

(1)beta-Actin: 838bp	
bac1b	5'-Z ATCTg gCACC ACACC TTCTA CAATg AGCTG CG-3'
bac2b	5'-CgTCA TACTC CTgCT TgCTg ATCCA CATCT gC-3'
(2)Glyceraldehyde-3-Phosphate Dehydrogenase (G3PDH): 983bp	
g3p1	5'-Z TgAAg gTCgg AgTCA ACggA TTTgg T-3'
g3p2	5'-CATgT gggCC ATgAg gTCCA CCAC-3'
(3)23-kDa Highly Basic Protein: 484bp	
23H1	5'-Z TAA ACA ggT ACT gCT ggg CCg gAA ggT G-3'
23H2	5'-CAC gTT CTT CTC-3'
(4)alpha-Tublin: 527bp	
atb1	5'-Z CAC CCg TCT TCA ggg CTT CTT ggT TT-3'
atb2	5'-CAT TTC ACC ATC Tgg TTg gCT ggC TC-3'
(5)Transferrin Receptor: 1347bp	
tfr1	5'-Z CCA CCA TCT Cgg TCA TCA ggA TTg CCT-3'
tfr2	5'-TTC TCA Tgg AAg CTA Tgg gTA TCA CAT-3'
(6)Ribosomal Protein S9: 431bp	
rp91	5'-gAT gAg AAg gAC CCA Cgg CgT CTg TTC g-3'
rp92	5'-gAg ACA ATC CAg CAg CCC Agg Agg gAC A-3'
(7)Hypoxanthine Guanine Phosphoribosil Transferase (HPRT): 467bp	
hpr1	5'-ggC gTC gTg ATT AgT gAT gAT gAA CC-3'
hpr2	5'-CTT gCg ACC TTg ACC ATC TTT ggA-3'

[0074] (During arrangement, guanine and C show cytosine, T shows thymine, and, as for A, Z shows the 5'-modification amino C6, as for adenine and G.)

The reaction product of about 400 to 1,300 bp of the size it was written clearly that performed PCR using these two primers by using as a mold Positive Control Amplified Fragment attached to the set, respectively is acquired.

[0075] The PCR reaction was performed on condition of the following using the DNA sir MARUSA salmon caviar by TAKARA SHUZO CO., LTD.

Reaction liquid: 50mM KCl10mM Tris-HCl (pH 8.4)

1.5mM(s) MgCl2 mold DNA The 2 5microl above-mentioned primer sets It is

0.25microMdNTPs respectively. It is 200microMTaqDNA Pori MERAZE (TAKARA SHUZO) respectively. 2.5 or more units was mixed and it was referred to as 100microl.

[0076] PCR cycle: -- like a DENACHURESHON fault -- : -- 94 degrees C like a 60-second annealing fault -- : -- 55 degrees C like a 120-second extension fault -- : -- 72 degrees C making 180 seconds or more into 1 cycle -- 30 cycle ******.

[0077] Agarose gel performed electrophoresis for 10micro of reaction liquid I generated at the above-mentioned PCR reaction 1.5%, and the fragment of about 400 to 1,300 bp was detected. Refining of a PCR product was performed using QIAquick of QIAGEN. The refined DNA probe (PCR product) is about 0.2. It dissolved in 3xSSC and used for the following operations so that it might become mg/ml concentration.

[0078] The fixation to the resin board of the PCR product used as a probe was first spotted using Nippon Laser & Electronics Lab. GTMASS Stamping equipment on the coating resin board which produced the probe nucleic acid solution of No.(1) - (7) in the above-mentioned example 1. Then, nucleic acid was made to fix on a resin board by the following operation. First, the spotted resin board is put into the airtight container into which the drier was put, and is saved at -20 degrees C overnight. A container is opened within the box prepared to 80% of humidity, and a resin board is dimmed. A resin board is used as a metal baseball bat, a spot side is turned upward, and it arranges, and prints with a 80-degree C dry heat machine for 1 hour. Next, UV crosslinker is used and UV irradiation is carried out at a resin board (65 mJ/cm2). It dips in blocking liquid for 20 minutes at 25 degrees C, putting a resin board into a slide rack and agitating it well. In addition, blocking liquid dissolves Succinic anhydride 3.3g in 195ml 1-methyl-2-pyrrolidinone, and mixes sodium borate (pH 8.0) of 1M with 15ml. Furthermore, 98 degrees C is dipped in sterile water for 2 minutes. After soaking a slide rack in ethanol 95%, taking it in and out 5 times finally and being air-dry at room temperature, it saves at DESHIKETA.

[0079] Fluorescence sign human liver origin polyA+ of the sample RNA by (Hybridization A) reverse transcription reaction with the DNA micro array produced in the fluorescence sign and example 9 of the example 10 sample RNA [RNA(made by CLONTECH) (1microg//micro / l) 1microl] random hexamer primer (1microg/mul) 5microl (5microg), DEPC-treated After 20microl Adding water, setting the whole quantity to 26microl and keeping it warm for 10 minutes at 70 degrees C, it cools in Hikami. To this solution, it is about 5 xfirst strand buffer (made by GIBCO BRL). 5microl and RNasin (made by Promega) for 10microl and DTT of 0.1M 1.5microl, dUTP of 1microl and 1mM for d(GAC) TP of 25mM(s) 2microl (final0.04mM), They are 2microl (final 0.04mM) and SuperScript about Cy3-dUTP of 1mM. RT 2.5microl is added for II (made by GIBCO BRL), and it is DEPC-treated further. A proper quantity of water(s) are added and it is made 50micro of whole quantity I.

[0080] After mixing slowly with a pipette and keeping it warm for 10 minutes at room

temperature, it is kept warm at 37 degrees C for 2 hours. 10microl is added for 5microl and NaOH of 0.1M in the solution after the end of a reaction, and EDTA (pH 8.0) of 0.5M is made to react to it for 10 minutes at 65 degrees C after mixture. Next, 20microl addition of 20microl and DEPC-Water is done [HCl of 0.1M] for 10microl and Tris-HCl (pH 7.5) of 1M. It is QIAGEN about this solution. It refined using QIAquick PCR purification Kit. The sterile water of 30microl performed elution.

(B) After adding 10microl and yeasttRNA 4mg/ml for Cot 1 DNA (1mg/(ml)) and adding 1microl for 1microl and pd(A)40-60 in the solution adjusted at the hybridization reaction (A), in it, it condensed at 2microl using the SpeedVac concentrator.

[0081] First, a pre hybridization is performed by the following operations. It ice-cools, after processing salmon testes DNA(1mg/(ml))50microl for 5 minutes at 95 degrees C. Sterile water is filtered by 165microl, and 10microl addition of the SDS is carried out 10%, and 150microl and 20 x Denhardt's soln. are filtered for 20x SSC with a 0.22-micrometer filter there, after mixing, 125microl and. Filtrate is annotated in the center of a cover glass (22 x 22 mm) by 15 lmicro. The DNA micro array produced in the example 9 is close brought from a cover glass, and a cover glass is stuck. A slide is turned to a table. It is kept warm at 65 degrees C for 1 hour. First, using a 500ml beaker, washing performs washing for 5 minutes twice by 400ml 2xSSC, and then performs washing for 5 minutes twice by 0.2xSSC. Next, it is sterile water, and rinses and a DNA micro array is air-dried.

[0082] Next, this hybridization is performed by the following operations. Sterile water 150microl and 20 x Denhardt's soln. for 215microl and 20x SSC 125microl, After carrying out 10microl addition of the SDS 10%, mixing and filtering with a 0.22-micrometer filter, it adds every [15micro / l] to the sample previously condensed below to 2microl, and is kept warm for 3 minutes at 95 degrees C. This mixed-solution is annotated in the center of a cover glass (22 x 22 mm) by 15 lmicro. The DNA micro array produced in the example 2 is close brought from a cover glass, and a cover glass is stuck. A slide is turned to a table. It is kept warm at 65 degrees C for 16 hours. First, using a 500ml beaker, washing performs washing for 5 minutes twice by 400ml 2xSSC+0.1% SDS, and then performs washing for 5 minutes by 0.2xSSC further for 5 minutes by 1xSSC. Next, it is sterile water, and rinses and a DNA micro array is air-dried.

[0083] The signal of a hybridization is a product made by Axon Instruments. The amount of fluorescence of each spot in the air-dry slide glass was measured using GenePix 4000A, and it evaluated by computing hybridization intensity. The result is shown in Table 4.

[0084]

[Table 4]

表 4

遺伝子名	ハイブリダイゼーション強度
(1)beta-Actin	50,000
(2)G3PDH	35,000
(3)23-kDa Highly Basic Protein	40,000
(4)alpha-Tublin	30,000
(5)Transferrin Receptor	3,000
(6)Ribosomal Protein S9	5,000
(7)HPRT	8,000
blank	2,000

[0085] The high value has come out of the hybridization signal intensity of a gene with the high amount of discovery reflecting the quantity, and in blank, a hybridization signal is low and it is possible to read a difference with a signal clearly.

[0086]

[Effect of the Invention] The coating resin board of this invention can fix nucleic acid, such as DNA, efficiently. As a result, the hybridization reaction of the nucleic acid which carried out the sign with the fluorescent substance etc. is carried out efficiently, the signal intensity of a sign can be measured in high sensitivity, and it becomes possible to analyze sample nucleic acid efficiently.

[0087] The resin board of this invention fits fixation of various substances, such as not only nucleic acid but peptide, protein, an antibody, etc., and also chip-izing, and micro array-ization.

[0088]

[Layout Table]

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[Translation completed.]